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Assessment of some fungal pathogens isolated from medicinal plants using different source of media

HAQEEQAT AFREEN ARSHI Dept.of Botany,Govt.Institute of Science Nipat Niranjan Nagar ,Caves Road,Auragabad,Maharashtra,India

SAHERA NASREEN Dept.of Botany,Govt.Institute of Science Nipat Niranjan Nagar ,Caves Road,Auragabad,Maharashtra,India

ABSTRACT

The mycelia growth rate, colony character and sporulation pattern of four fungal isolates, grown on four different culture media viz., potato Dextrose Agar (PDA), Nutrient Agar (NA), Corn Meal Agar (CMA), Sabaouraud Agar (SDA), were observed after seven days of incubation at 250C. The colony diameter, colony characters [texture, Surfacecolour, Form, Elevation] and sporulation of selected test fungi were greatly influenced by the type of growth medium used. CMA and PDA exhibited comparatively higher mycelia growth in four test fungi.

KEYWORDS Mycelial growth, colony character, sporulation, culture media.

Medicinal plants form a numerically large group of economically important plants which provide basic raw material for medicines. Medicinal plants are those plants which are rich in secondary metabolites & are potential source of drugs. These secondary metabolites includes alkaloids, glycosides, caumarins etc.

The plants have been used to cure diseases since antiquity. The ancient Indians were having a vast knowledge of medicinal plants. This is evident from the ancient treatise such as Materia Medica, Rigveda, Nighatus & koshas.

Considerable revival of the use of plants for medical purpose has taken place through out the world, because of common feeling that anything from nature is safer to synthetics. For the sustained supply of the raw materials pharmaceutical industries and preventing them from becoming extinct, these days major emphasis is being laid on their cultivation.

Like any other plants, medicinal plants too have to bear the attacks of injurious pests such as Fungi, bacteria, viruses etc.

In laboratory fungi are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical & physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology pigmentation and sporulation depending upon the composition of specific cultur medium, pH, temperature, light water availability and surrounding atmospheric gas mixture [Northolt and Bullerman, 1982; Kuhn and Ghannoun, 2003 kumara and Rawal, 2008].

Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelia growth and sporulation on artificial media are important biological characteristics [St. Germain and Summerbell, 1996] with these perspectives, the present study was undertaken to observe the assessment of fungal pathogens using different source of Media.

Material and Methods :

Collection of medicinal plants :

Different infected parts of medicinal plants such as infected leaves of tulsi *[Ocimum tenuiflorum],* infected bulbs of garlic *[Allium sativum],* infected fruits of papaya *[Carica papaya]* were collected from Botanical garden of Govt. Institute of science and various parts of Aurangabad city, Maharashtra, India.

Isolation of fungi :

Isolation of fungi was carried out by surface sterilization method [kinkel and Andrews, 1988]. Twentifive Bits [5 x 5 mm] of each infected part of medicinal plants were submerged in 70% ethanol for 1 min, then transferred into 15% H_2O_2 for 1 min and again kept in 70% ethanol for 1 minute. Thereafter, the bits were serially washed in 10 times in sterile distilled water, blotted dried and then placed in moist chambers for about 3 days by enriching the bits with nutrient medium using glucose as source after that fungus were transferred in each of four petriplates containing Potato Dextrose Agar [PDA] medium supplemented with streptomycin [100 mg / l] and incubated at 25 1^0C for 7 days. The fungal colonies were isolated in fresh sterilized petri plate containing PDA and were identified. In this way *Alternaria alternata* was isolated from infected leaves of tulsi *[Ocimum tenuiflorum], Aspergillus niger* was isolated from infected bulb of garlic *[Allium sativum], Fusarium solani* was isolated from infected fruit of Papaya *[carica papaya]* and *Rhizopus nigricans* was also isolated from infected fruit of Papaya *[carica papaya].* These four fungi were selected and 5mm discs of each fungus obtained from pure cultures were transferred at the centre of sterile petridishes (in triplicates) containing four different media viz. Potato Dextrose Agar [PDA], Corn Meal Agar [CMA] , Nutrient Agar [NA] and Sabouroud Agar[SDA].

Preparation of Media :

Potato Dextrose Agar [PDA] - Potato (Peeled) 200g, Dextrose 20g, Agar 20g, Distilled H_2O 1L.

Corn Meal Agar [CMA] - corn (fresh) 200g, Dextrose 20g, Agar 20g, Distilled H_2O 1L.

Nutrient Agar [NA] - Glucose 10g, Peptone 10g, Beef extract - 03g, Sodium Chloride 5g, Agar 15g, Distilled H_2O 1 L.

Sabouroud Agar [SDA] - Dextrose 40g, peptone - 10g, Agar 20g, Distilled H_2O 1L.

The pH of the test media was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi. The petri plates were then incubated for 7 days at 25^0C in BOD incubator and colony character of such fungus was recorded. Sporulation was assessed on glass slides by mounting a small

portion of mycelia in Lactophenol - cotton the stain and Observed under microscope.

Result and Discussions :
All four culture media supported the growth of test fungi to various degrees.

Rate of growth of *Alternaria alternata* **in different culture media :**

Alternaria alternata exhibited higher colony growth on PDA colony was completed in 7 days with heavy sporulation and colony appeared as dark green coloured, circular in form with flat elevation. On SDA it showed slightly slow growth. Colony was completed in 9 days with moderate sporulation and colony appeared as gray green & velvety thick texture. On NA also it showed moderate sporulation and appeared as olive green with flat elevation.(Table-1,fig.4)

Rate of growth of *Fusarium solani* **in different culture media :**
Fusarium solani exhibited higher colony growth on PDA followed by SDA while exhibited lower colony growth on NA. On PDA colony was completed in 7 days with heavy sporulation. Surface colour changes from white to light pink and colony appeared as velvety thick, circular with flat elevation. On SDA colony war completed in 7 days with heavy sporulation surface colour is white, velety thick texture with imbonate elevation. On CMA colony was completed in 9 days with moderate sporulation, Irregular in form with flat elevation. On NA colony growth was slow, colony completed in 11 days with poor sporulation. (Table-1,fig.2).

Growth of *Aspergillus niger* **on different culture media :**
In all four culture media growth of *Aspergillus niger* was good. However it exhibited higher colony growth on PDA. Colony was completed in 7 days with heavy sporulation. On SDA colony was completed in 7 days with moderate sporulations and irregular in form with imbonate alluation on NA colony was completed in 7 days with moderate sporulation and circular in form with flat elevation. (Table-1,fig.3).

Growth of *Rhizopus nigricans* **on different Culture media :**
Rhizopus nigricans exhibited high colony growth on all four culture media. In all media types colony was almost completed in 3 days with heavy sporulation and circular in form and with flat elevation.(Table-1,fig.1).

In Present study, Texture, Elevation pattern and form observed in fungal colonies were found to be influenced by the culture media. Type of culture media and their chemical compositions significantly affected the mycelia growth rate and conidial production of phoma exigua [Zhae and simon, 2006].

PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelia growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelia growth [Xu et al., 1984; Maheshwari et al,1999; saha et al., 2008].

The fungal systematic is still based mainly on morphological criteria as observable characteristics. Hence, fungi are recognized and identified basically by their phenotypes [Zain et al., 2009]. Morover the variation in calour of spores, especially among Aspergillus, is one of the main criteria used widely for identification and taxanomic placement [st. Germain and Summerbell, 1996] which seems to b e mainly attributed to the constituents of a medium.

Conclusion :
The media components are an important criteria for fungal culture and study, In present study, type of culture media and their chemical composition significantly affected the mycelia grouth rate and conidial production in all tested fungi viz. *Alternaria alternata, Fusarium solani Aspergillus niger, Rhizopus nigricans*. Our finding revealed that Corn Meal Agar [CMA] was most suitable for heavy sporultion while Potato Dextrose Agar [PDA] reproduced most visible colony morphology.

Table I. Mycelia growth, colony characters & sporulation pattern of fungal isolates on four culture media after 7 days.

Fungi	Media type	Colony diameter (cm)	Colonycharacters					Sporulation
			Texture	Surface colour	Form	Elevation	no.of days. for Completion	
Alternaria alternata	CMA SDA PDA NA	8.9 9.0 9.00 8.8	Fine Velvetythick Fine Fine	Darkgreen Graygireen Gieen Olivegreen	Circular Circular Circular Circular	Imbonate Imbonate Imbonate Flat	7 9 7 9	Heavy Moderate Heavy Moderate
Fusarium Solani	CMA SDA PDA NA	9.1 8.9 9.0 8.7	Fine Velvetythick Velvetythick fine	white white to Pink White to pink Light pink	Irregular Circular Circular Irregular	Flat Imbonate flat Flat	7 7 7 11	Heavy Heavy Heavy Poor
Aspergillus niger	CMA SDA PDA NA	8.5 8.4 8.7 8.9	Powdery Powdery Dense velvety Powdery	White mycellum with black spores white with black spores Hyaline with black spores	Irregular Irregular Irregular Circular	Flat Immolate Flat Flat	7 7 7 7	Heavy Heavy Heavy moderate
Rhizopus nigzicans	CMA SDA PDA NA	9.1 9.0 9.1 9.0	Fine Fine Fine Fine	White White White White	Circular Circular Circular Circular	Flat Flat Flat Flat	3 4 3 3	Heavy Heavy Heavy Heavy

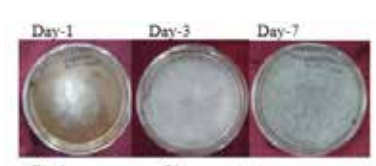

Fig.1 *Rhizopus nigricans*


Fig.2 *Fusarium solani*

Fig.1 *Rhizopus nigricans*


Fig.2 *Fusarium solani*

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